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Feature Article

Top-Down and Bottom-Up Approaches in Phytoplasma Research: Advancing Pathogenesis, Taxonomy, and Diagnostics

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Abstract

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Phytoplasmas are minute, cell wall-less bacteria responsible for devastating plant diseases, leading to significant economic loss in agriculture. This article explores recent advancements in phytoplasma research, focusing on pathogenesis, taxonomy, and diagnostics through top-down and bottom-up approaches. Top-down multi-omics studies have provided a system-level understanding of phytoplasma-induced disruptions, particularly in sugar metabolism and hormone signaling, revealing their extensive impact on plant physiology. Complementing this, bottom-up strategies have dissected molecular interactions, elucidating how phytoplasmas derail meristem fate, modulate plant growth patterns, alter plant architecture, and induce characteristic symptoms. Advances in taxonomy and classification have improved species differentiation, integrating 16S rRNA sequencing, multilocus sequence typing (MLST), and whole-genome sequencing (WGS), with database-guided tools refining classification accuracy. The development of cutting-edge diagnostic technologies, such as clustered regularly interspaced short palindromic repeats-based (CRISPR-based) detection, has significantly enhanced the sensitivity, specificity, and efficiency of phytoplasma identification and surveillance. Additionally, the integration of big data analytics and AI-driven models has pioneered image-based symptom recognition, supporting disease surveillance and monitoring.

Key words: Meristem fate derailment, *i*PhyClassifier, Artificial intelligence (AI), Omics, Floral transition.

INTRODUCTION

Phytoplasmas are small bacteria that infect a wide range of plants, causing severe economic loss worldwide. Residing in plant phloem tissues and being adapted to the nutrient-rich environment, phytoplasmas have gone through reductive evolution, as manifested by the lack of many genes that are involved in metabolic pathways essential to free-living organisms (Oshima *et al*. 2004; Kube *et al*. 2012; Tan *et al*. 2021). In nature, phytoplasmas are transmitted by phloem-feeding insect vectors, primarily leafhoppers, planthoppers, and psyllids, which acquire the pathogen while feeding infected plants and spreading it to healthy ones. This vector-mediated transmission enables phytoplasmas to persist in ecosystems and infect a wide range of plant species, often leading to devastating outbreaks in both agricultural and natural environments (Weintraub & Beanland 2006). Phytoplasmas cannot be cultured *in vitro* (Wei & Zhao 2022), presenting a significant challenge for research, diagnostics, and disease management.

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Phytoplasma-infected plants exhibit a diverse array of symptoms, including virescence (greening of floral organs), phyllody (conversion of floral structures into leaf-like formations), witches'-broom (WB) (excessive shoot proliferation), yellowing, and stunted growth (Wei *et al*. 2013, 2022). These physiological disruptions can severely impact plant development and productivity, leading to economically significant diseases such as grapevine yellows, date palm dieback, and apple proliferation (Seemüller & Schneider 2004; Belli *et al*. 2010; Gurr *et al*. 2016).

Beyond their detrimental effects, phytoplasmas can induce unusual yet visually striking morphological changes, which are sometimes considered beneficial in horticultural settings. For example, phytoplasma-associated poinsettias exhibit a branching phenotype, which is more attractive in aesthetics (Lee *et al*. 1997). The ability of these pathogens to manipulate plant development raises a fundamental question: What molecular and cellular mechanisms drive these alterations in plant morphology? Understanding these mechanisms is essential for unraveling phytoplasma-host interactions and developing effective disease control strategies.

A systematic and integrative research approach is necessary to investigate the complexity of phytoplasma-induced morphological changes. This involves top-down and bottom-up methodologies (Shahzad & Loor 2012). The top-down approach, often applied through high-throughput omics studies, provides a broad overview of host responses at the genomic, transcriptomic, proteomic, and metabolomic levels (Tan *et al*. 2021, 2025). This comprehensive perspective helps identify global changes in gene expression and biochemical pathways that occur upon phytoplasma infection. In contrast, the bottom-up approach focuses on specific molecular players, including key regulatory genes, proteins, and signaling molecules. Research using this approach has identified meristem-regulating genes and floral organ identity genes that undergo disruption upon infection, leading to developmental reprogramming (MacLean *et al*. 2011; Sugio *et al*. 2011; Wei *et al*. 2013, 2019, 2022). Additionally, several phytoplasma-derived effector proteins have been identified as key factors in manipulating plant growth by targeting host regulatory pathways (Hoshi *et al*. 2009; MacLean *et al*. 2011; Sugio *et al*. 2011; Maejima *et al*. 2014; Huang *et al*. 2021).

These complementary approaches enhance the understanding of how phytoplasmas alter plant architecture and provide valuable insights into plant-pathogen interactions. By deciphering these molecular mechanisms, future strategies can be developed to mitigate the negative impacts of phytoplasma diseases while exploring potential applications for controlled morphological modifications in agriculture and horticulture.

Phytoplasma taxonomy has transitioned from early misclassification to a molecular-based framework, addressing challenges associated with their unculturable nature (Wei & Zhao 2022). The *Candidatus* Phytoplasma designation, along with whole-genome sequencing (WGS), average nucleotide identity (ANI), and multilocus sequence typing (MLST), has refined species delineation and classification (Bertaccini *et al*. 2022; Wei & Zhao 2022). The *i*PhyClassifier database further standardizes strain identification, automating classification and expanding reference datasets (Zhao *et al*. 2009; Wei & Zhao 2022). Advances in molecular diagnostics, including polymerase chain reaction-based (PCR-based) methods (Lee *et al*. 1993), loop-mediated isothermal amplification (LAMP) (Dickinson 2015), and clustered regularly interspaced short palindromic repeats-based (CRISPR-based) detection (Wheatley *et al*. 2022), have improved disease surveillance and management.

AI and data science are introducing new approaches to phytoplasma research, particularly in disease monitoring and classification. AI-driven image analysis using convolutional neural networks (CNNs) shows promise in assisting disease detection, while databases such as *i*PhyDSDB and the Phytoplasma Disease and Classification Database enhance disease rec-

ognition and classification (Wei *et al*. 2024a, 2024b). Big data analytics are also improving the understanding of disease patterns, aiding more informed management strategies. Ongoing research into phytoplasma biology, taxonomy, and disease monitoring, combined with advancements in molecular diagnostics, AI, and big data analytics, may further enhance disease detection and management in agriculture.

PHYTOPLASMA-INDUCED ALTERATIONS OF PLANT GROWTH PATTERNS AND ARCHITECTURE

Sexual reproduction is a key process in the life cycle of seed plants, occurring in three main phases: flowering, fruit formation, and seed dispersal, followed by germination (Lord & Russell 2002). The transition to flowering marks the shift from vegetative to reproductive growth, regulated by genetic programs and environmental cues such as light, temperature, and hormones. During this stage, the shoot apical meristem transforms, with the vegetative meristem (VM) converting into an inflorescence meristem (IM), which generates floral meristems (FMs). These FMs develop into sepals, petals, stamens (male reproductive organs), and carpels (female reproductive organs containing ovaries and ovules) (Kater *et al*. 2006). Once carpels form, FMs cease further meristematic activity.

Pollination, facilitated by wind, water, or pollinators, transfers pollen from the stamen to the carpel, where fertilization occurs, initiating seed and fruit development (Seymour *et al*. 2013). The fruit protects seeds and aids in their dispersal through various mechanisms. Dormant seeds remain inactive until favorable conditions trigger germination, reactivating metabolic processes and stimulating root and shoot apical meristems for new growth (Koornneef *et al*. 2002). From flowering to germination, this cycle is tightly regulated by genetic pathways and environmental factors, ensuring reproductive success and species survival (De Kroon *et al*. 2005; Barthélémy & Caraglio 2007). In healthy plants, once the floral transition is complete, the formation of flowers, fruits, and seeds follows a predetermined, irreversible process. However, in plants infected by phytoplasmas, this natural progression is disrupted, leading to distinct abnormalities such as virescence, phyllody, and arrested inflorescence, leading to a significant reduction in fruit set and seed formation (Wei *et al*. 2013, 2019).

A bottom-up approach was employed to explore the mechanism underlying these morphological alterations, focusing on Columbia Basin potato purple top (PPT) phytoplasma and its alternate host, the tomato (the model pathogen-host pair our group is utilizing for interaction studies).

Small seedling graft inoculation

The experimental plants were established using a graft inoculation technique. PPT phytoplasma was introduced into small tomato seedlings at the four-leaf stage (rootstock, Wei *et al*. 2013, 2019). Infected shoots displaying WB symptoms were used for the phytoplasma inocula (scions). This small-seedling grafting method allowed for a controlled infection process, providing a clear timeline for symptom progression and enabling researchers to observe the sequential development of phytoplasma-induced symptoms. The study challenged the previous assumption that phytoplasmas cause unique, host-specific symptoms. Instead, the findings demonstrated that a single phytoplasma strain can induce distinct symptoms depending on the meristematic stage it infects, highlighting the critical role of meristem fate in determining the nature of phytoplasma-induced abnormalities.

Phytoplasma infection-induced meristematic derailment

Phytoplasma infections exert their effects by disrupting meristems responsible for cell division and differentiation in plants. The specific developmental outcome of infection depends on whether the vegetative meristem, inflores-

cence meristem, floral meristem, or flower organ primordia are affected (Fig. 1, Wei *et al*. 2013). The floral transition follows a sequential shift in meristematic fate, progressing from the vegetative meristem to the inflorescence meristem, then to the floral meristem, and finally to flower organ primordia formation. However, when phytoplasmas infect plants, the disruption initially affects the later stages of floral transition, as the infection occurs after the process has already begun, primarily targeting the floral meristem and flower organ primordia. As symptoms progress, the infection gradually extends to earlier stages, including the inflorescence meristem and the vegetative meristem, leading to broader developmental abnormalities (Wei *et al*. 2013, 2019).

In a normal flowering tomato line, 28 d post inoculation (dpi), PPT phytoplasma infection directly affects the floral meristem. The development of flowers is prematurely terminated, leading to the formation of flattened floral meristems. This results in the big bud (BB) phenotype, where sepals enlarge abnormally, and petals, stamens, and carpels are underdeveloped and fail to develop into fully formed flowers (Fig. 1A).

At 45 dpi, PPT phytoplasma infection affects the inflorescence meristem, arresting the initiation of floral meristems. Instead of forming individual flowers, the plant continuously produces repetitive inflorescence meristems, resulting in a cauliflower-like inflorescence structure (CLI, Fig. 1B). This disruption suggests that phytoplasma alters the delicate regulatory balance between meristem maintenance

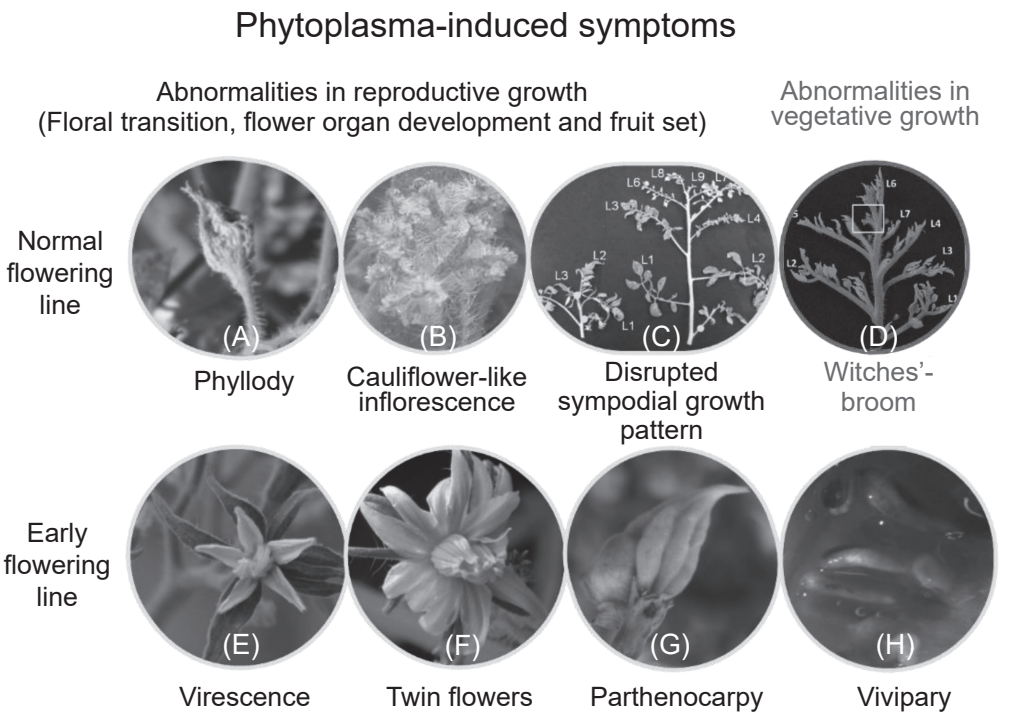

Fig. 1. Symptoms of potato purple top (PPT) phytoplasma infection in tomato plants showing abnormalities in vegetative and reproductive growth. (A–C) Abnormalities in reproductive growth in normal flowering tomato line (flower after 10–12 leaves). (A) Big bud (BB) or phyllody; (B) cauliflower-like inflorescence (CLI); (C) disrupted sympodial growth pattern (DSGP); (D) PPT phytoplasma-induced alterations in vegetative growth. Witches'-broom (WB) is characterized by dense clustering of shoots due to abnormal branching. (E–H) Abnormalities in reproductive growth in early flowering tomato line (flower after 3–5 leaves, E–H). (E) virescence; (F) twin flowers; (G) parthenocarpy; (H) vivipary.

and differentiation, preventing proper floral development.

When PPT phytoplasma infection targets the vegetative meristem, the transition from vegetative to reproductive growth is delayed, resulting in an extended vegetative phase with the production of excess leaves. Instead of three leaves per sympodium unit, more leaves are produced in PPT phytoplasma-infected plants. This phenomenon is called "disrupted sympodial growth pattern" (DSPG, Fig. 1C).

An early flowering tomato line, which overexpresses the Single Flower Truss (SFT) gene and flowers after producing three to five leaves (Lifschitz *et al*. 2006; Shalit *et al*. 2009), is also used for PPT phytoplasma infection. SFT, the tomato ortholog of Flowering Locus T (FT) in *Arabidopsis thaliana*, encodes florigen, a mobile signaling molecule that promotes flowering. By performing graft inoculation at the four-leaf stage combined with an early flowering line (35S: SFT), this setup provided an extended time window for phytoplasma-plant interactions, particularly during critical developmental phases of floral transition. As a result, more developmental abnormalities in flower, fruit, and seed formation are observed (Fig. 1E–G). Some plants develop virescence, where floral organs, especially petals, turn green (Fig. 1E), and twin flowers, where flower organs are duplicated (Fig. 1F). Another common abnormality is parthenocarpy, where fruits develop without fertilization (Fig. 1G). Additionally, very few plants set fruit, and some exhibit vivipary, where seeds begin germinating while still inside the fruit (Fig. 1H). This phenomenon indicates that phytoplasma infection disrupts seed dormancy mechanisms, possibly by altering abscisic acid (ABA) levels, which generally prevent premature germination. As symptoms progress, phytoplasma infection can primarily affect the early stages of floral transition; therefore, virescence, twin flowers, parthenocarpy, and vivipary are no longer observed; instead, like the normal flowering line, only BB and CLI remain detectable.

Gene regulation and functional studies

In phytoplasma-infected plants, genes involved in meristem maintenance and transition, including *SFT*, *FA*, and *AN*, as well as floral organ identity genes (*MC*, LePI, *TAP3*, *TAG*, and *TM5*) and those regulating floral organ primordia development, were found to be misregulated. These observations were made using gain- and loss-of-function tomato lines to dissect the regulatory impacts of infection. The data indicated that phytoplasma infection leads to the downregulation of inflorescence and floral meristem identity genes while simultaneously upregulating genes involved in meristem reiteration, explaining the prolonged vegetative growth, loss of floral determinacy, and floral malformations observed in infected plants (Wei *et al*. 2013, 2019).

Based on these findings, a working model was developed to explain how phytoplasma infection disrupts plant meristem function, leading to diverse developmental abnormalities. This model suggests that phytoplasma manipulates gene regulatory networks in three main ways: delaying floral transition by suppressing flowering genes, arresting floral meristem initiation by disrupting meristem maintenance pathways, and altering floral organ identity by misregulating homeotic gene expression (Wei *et al*. 2013, 2019).

PHYTOPLASMA-INDUCED ALTERATION IN VEGETATIVE GROWTH (WITCHES'-BROOM)

In addition to the symptoms tied to plant reproductive growth, phytoplasma-induced symptoms include a typical abnormality called "witches'-broom" (WB, Fig. 1D), which specifically affects the plant's vegetative growth. This branching arises from each leaf axil and further develops axillary buds, resulting in an excessive, broom-like appearance (Wei *et al*. 2013, 2022). While shoot branching is observed in plants infected by viruses, viroids, and fungi (Satoh *et al*. 2013; Kaur & Kaur 2018; Bao *et al*. 2019; de Haro *et al*. 2019), phytoplasma-in-

duced branching tends to be more severe. The underlying cause of this abnormal branching is suspected to be the disruption of plant sugar metabolism and hormonal balance due to the pathogen's presence.

Our top-down omics findings also indicate that phytoplasma infection leads to significant alterations in the sugar metabolism of host plants (Details, please refer to the next section). To elucidate the mechanism underlying the formation of WB, the bottom-up approach focusing on starch and sucrose was employed. A significant starch buildup was observed in PPT phytoplasma-infected leaf samples using Lugol's iodine staining (Wei *et al*. 2022). Under transmission electron microscopy (TEM), irregular and swollen starch granules were also found. This TEM image showed that the swelling starch granule occupied almost all the chloroplast, leading to damage to the chloroplast. This phytoplasma-induced blockage of starch breakdown triggers autophagy to degrade the injured chloroplasts (Wei *et al*. 2022; Inaba *et al*. 2023a).

The sucrose content in different plant tissues was measured, and the distribution of auxins and cytokinins was visualized using the reporter tomato lines, including DR5 (direct repeat 5)::GUS (β-glucuronidase) and ARR5 (arabidopsis response regulator 5)::GUS lines (Wei *et al*. 2022). The expression profile of genes involved in lateral bud initiation and outgrowth was assessed. The sucrose level was higher in source leaves and leaf axils and lower in stem and root (Wei *et al*. 2022). This indicates phytoplasma infection disrupts phloem translocation of sucrose, and this is caused by excessive callose deposition (Fig. 2). Aniline blue staining revealed that more callose was found in infected phloem compared to healthy phloem (Fig. 2A and B). Using TEM, it is evident that callose depositions sealed the sieve pores of infected plants (Fig. 2C and D). Our results showed that

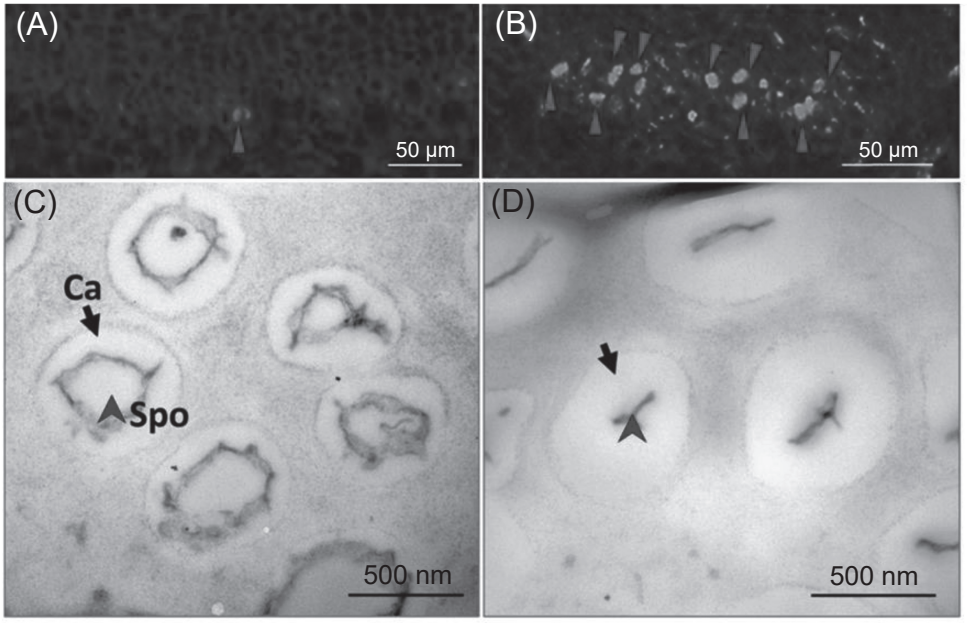

Fig. 2. Callose deposition in the tomato plants induced by potato purple top (PPT) phytoplasma. (A, B) Aniline blue staining of cross-stem sections of (A) mock control and (B) infected plants. Red triangles show the phloem's callose deposition (bright blue fluorescence). Scale bar = 50 μm. (C, D) Transmission electron microscopy (TEM) analysis revealed excessive callose deposition in the sieve plates of tomato plants induced by PPT phytoplasma. Blue arrowheads point to the sieve plate and sieve pore (Spo), respectively. Black arrows indicate callose (Ca) deposition. Scale bar = 500 nm. This figure is attributed to https://doi.org/10.3390/ijms23031810. Reproduced according to the terms of the Creative Commons Attribution License.

sucrose accumulation in leaf axils triggers the initiation of axillary buds. This process is complemented by the involvement of cytokinins, as evidenced by their distinct distribution in leaf axils (Fig. 3, Wei *et al*. 2022).

Moreover, the findings indicate that phytoplasma infection leads to early leaf senescence, and the marker genes that induce leaf early senescence were significantly upregulated. Phytoplasma-induced blockage of starch breakdown down-regulated expression of genes involved in gibberellin synthesis. Gibberellin is a well-known plant hormone that contributes to plant height. This is why the stunted growth was observed in phytoplasma-infected plants. Expression patterns of genes associated with axillary bud outgrowth, such as *BRC1* and *SPLs*, change infection, contributing to shoot branching (Wei *et al*. 2022).

Based on these findings, we proposed a model (Fig. 4), which demonstrates that phytoplasma-induced WB symptom is driven by a multitude of mechanisms, including the blockage of starch breakdown, chloroplast degradation, premature leaf senescence, sucrose reallocation, and cytokinin redistribution. As a result, infected plants exhibit leaf chlorosis, reduced leaf size (little leaf), and stunted growth. Furthermore, phytoplasma infection disrupts sugar metabolism and interferes with sucrose transport, causing sucrose to be redirected to leaf axils. This redistribution coincides with an increased cytokinin concentration, promoting axillary bud initiation. The continuous cycle of axillary bud formation and excessive outgrowth, coupled with accelerated leaf senescence, ultimately manifests as WB symptoms.

"OMICS" STUDIES ON PHYTOPLASMAS AND INFECTED PLANTS

The top-down approach was employed to study interactions between phytoplasmas and plants, including widely used genomics, transcriptomics, proteomics, and metabolomics (Tan *et al*. 2021), as well as additional derivative omics such as ubiquitomics, lipidomics, and volatilomics (Inaba *et al*. 2023b; Ivanauskas *et al*. 2023).

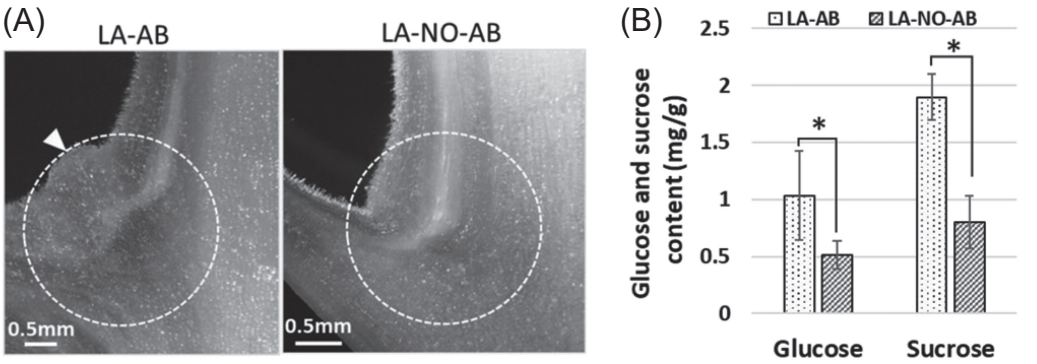

Fig. 3. Distribution of cytokinin-responsive signal and sugar content in leaf axils of ARR5 (arabidopsis response regulator 5)::GUS (β-glucuronidase) cytokinin reporter tomato plants infected with potato purple top (PPT) phytoplasma. (A) Cytokinin activity, as indicated by GUS staining, was analyzed in leaf axils of ARR5::GUS reporter tomato plants infected with PPT phytoplasma. Intense GUS staining, reflecting a high level of cytokinin, was observed in leaf axils where a new axillary bud had been initiated (LA-AB, left panel). In contrast, light GUS staining, indicating a lower cytokinin signal, was detected in leaf axils where no axillary bud developed (LA-NO-AB, right panel). The newly initiated bud is marked by a white triangle. Leaf axils (LA-ABs and LA-NO-ABs) highlighted with white circles were collected for sugar content analysis. Scale bar = 0.5 mm. (B) Glucose and sucrose levels were measured in the sampled leaf axils of PPT phytoplasma-infected plants. Significantly higher glucose and sucrose contents were detected in LA-ABs compared to LA-NO-ABs ($^{*}P < 0.01$), suggesting a correlation between cytokinin accumulation, sugar availability, and axillary bud initiation. This figure is attributed to https://doi.org/10.3390/ijms23031810. Reproduced according to the terms of the Creative Commons Attribution License.

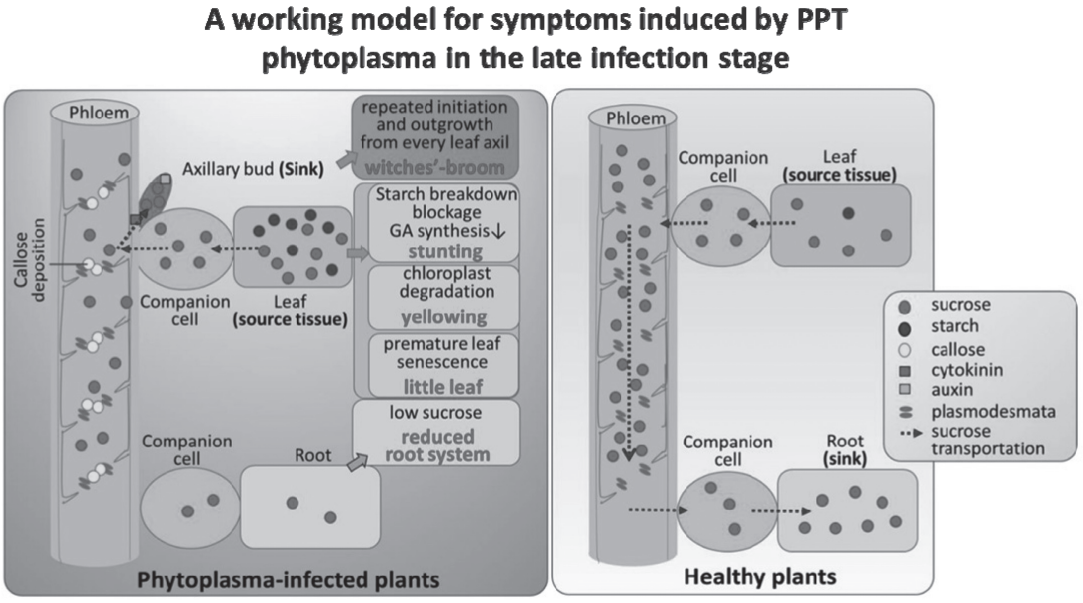

Fig. 4. A proposed working model for symptoms induced by potato purple top (PPT) phytoplasma in the late infection stage. In this model, PPT phytoplasma infection disrupts starch breakdown, leading to the degradation of damaged chloroplasts and premature leaf senescence. As a result, symptoms such as little leaf and leaf chlorosis emerge. The blockage of starch breakdown also suppresses gibberellin (GA) synthesis, restricting plant growth and reducing height. Additionally, PPT phytoplasma infection causes excessive callose deposition, reducing sieve pore size and impeding sucrose translocation through the phloem. This leads to sucrose accumulation in leaf axils, which, in turn, triggers axillary bud initiation. Cytokinin distribution in leaf axils contributes to this process, while auxin promotes the elongation of the axillary buds. Repetitive axillary bud initiation and outgrowth lead to witches'-broom formation. This figure is attributed to https://doi.org/10.3390/ijms23031810. Reproduced according to the terms of the Creative Commons Attribution License.

Genomic characteristics of phytoplasmas

Phytoplasma genomes are small and adenine-thymine-rich (AT-rich), with sizes ranging from 530 to 1,200 kb (Tan *et al*. 2021; Wei & Zhao 2022). Their reduced genome size results from extensive gene loss, a characteristic common among obligate intracellular pathogens. A defining feature of phytoplasma genomes is the presence of clustered, multiple-copy genes, forming a distinctive genomic architecture known as sequence-variable mosaics (SVMs) (Jomantiene *et al*. 2007). These structures are highly dynamic, repetitive sequences that exhibit high variability across different phytoplasma strains. The presence of these mosaics suggests an evolutionary mechanism that allows phytoplasmas to maintain genetic diversity despite their reduced genome size. This flexibility may play a critical role in their ability to adapt to different hosts, evade plant immune responses, and manipulate host physiology. These variable genomic regions have also been described as potential mobile units (PMUs) (Bai *et al*. 2006) or mobile unit genes (MUGs) (Arashida *et al*. 2008). The terminology reflects their suspected role in genomic plasticity, where mobile genetic elements facilitate genetic recombination, allowing phytoplasmas to acquire or modify new functions. These mobile units may contribute to the horizontal exchange of genes among phytoplasma strains, promoting adaptability in response to environmental pressures and interactions with diverse host species.

The origin of these mobile elements remained a mystery until their relationship to prophages was discovered (Wei *et al*. 2008a).

This finding linked the presence of SVMs and PMUs to bacteriophage-derived genetic material, suggesting that horizontal gene transfer via phage activity has significantly shaped phytoplasma genomes. Despite variations in genome sizes, after removing the prophage sequence, the genomes of all phytoplasmas were similar in size, around 550 to 580 kb, akin to the circular chromosome of *Mycoplasma genitalium*, known for its minimal gene set for cellular life (Zhao *et al*. 2014). No SVMs were observed in acholeplasmas, spiroplasmas, or mycoplasmas, which share a close phylogenetic relationship with phytoplasmas. However, all phytoplasmas possess SVMs. This discovery implies that phage-mediated recombination has played a fundamental role in the evolution of phytoplasmas, introducing new genetic material that may enhance virulence, host adaptation, and transmission efficiency. Phytoplasmas likely acquired these mobile elements through repeated cycles of phage infection, allowing them to incorporate and retain beneficial genetic fragments over time.

Another common trait within phytoplasma genomes is the absence of various genes related to metabolic pathways, including the tricarboxylic acid cycle, pentose phosphate pathway, sterol biosynthesis, fatty acid biosynthesis, *de novo* nucleotide synthesis, and biosynthesis of most amino acids (Oshima *et al*. 2004). However, phytoplasma genomes harbor multiple copies of transporter-related genes, such as ABC transporters, that can import nutrients into the cell. This indicates that phytoplasmas are highly dependent on metabolites imported from the hosts for their own growth and infection (Oshima *et al*. 2004).

Phytoplasma infection-induced metabolic reprogramming in plants

Phytoplasma infection induces significant metabolic reprogramming in host plants, leading to extensive alterations in primary and secondary metabolic pathways. By utilizing sweet cherry trees and sweet cherry virescence (SCV) phytoplasma, the metabolomics study identified 676 metabolites, with 187 differentially expressed metabolites. The majority of these belonged to categories such as carbohydrates, fatty acids/lipids, amino acids, and flavonoids, indicating a broad metabolic shift in response to infection. These findings suggest that phytoplasma systematically manipulates host metabolism to optimize conditions for its survival and proliferation (Tan *et al*. 2021).

One observation was the upregulation of glycolysis and pentose phosphate pathway (PPP) activity, which provides the energy and building blocks necessary for phytoplasma growth and replication. The infection-induced increase in glucose-6-phosphate, sedoheptulose 7-phosphate, and maltotetraose suggests a breakdown of carbohydrates (Tan *et al*. 2021). By promoting these pathways in the plant, phytoplasma ensures a steady influx of energy-rich compounds and metabolic intermediates, which are likely to sustain its proliferation.

Increased accumulation of several key sugars, such as D-glucose, D-glucose-6-phosphate, and D-sedoheptulose-7-phosphate, indicates a shift in source-to-sink relationships within the plant, potentially redistributing resources to favor the pathogen's needs. This metabolic reprogramming is consistent with previous transcriptomic studies showing upregulation of genes related to sugar transport and metabolism in phytoplasma-infected plants (Tan *et al*. 2019). Furthermore, the detection of melezitose, a sugar known to attract insect vectors, suggests that phytoplasma infection may also indirectly facilitate its own spread by modifying plant metabolism to enhance insect feeding behavior.

Transcriptomic insights into phytoplasma-induced symptoms (little leaf formation within WB structures)

The transcriptomic changes underlying the formation of little leaves within WB structures in sweet cherry trees infected by Sweet Cherry Virescence (SCV) phytoplasma were investigated. This research focuses on how phytoplasma infection alters plant growth, particularly in terms of leaf size reduction and premature se-

nescence (Tan *et al*. 2025). Morphological analysis revealed that infected leaves were smaller, yellowed, and irregularly shaped, with reduced palisade cell numbers and phloem lumen size, impairing expansion and sugar transport. Hormonal imbalances, including increased abscisic acid (ABA) and jasmonic acid (JA) but reduced cytokinins, contributed to growth inhibition and premature senescence.

Transcriptomic analysis revealed extensive changes in gene expression, particularly in pathways involved in ribosome biogenesis, DNA replication, and cell cycle progression. Many genes related to ribosome formation and protein synthesis were significantly downregulated, leading to an overall suppression of cell division. The downregulation of key cyclin genes (CYCD3; 1 and CYCD6; 1) suggests that phytoplasma infection causes a cell cycle arrest at the G1 phase, preventing proper leaf expansion and growth. Despite these disruptions, genes involved in photosynthesis and sugar metabolism were upregulated, likely as a compensatory response to the plant's energy deficit. However, this increased gene expression did not translate into higher sugar availability, as transport and utilization remained impaired (Tan *et al*. 2025).

Further analysis indicated that little leaf formation might be associated with premature leaf senescence. Marker genes associated with senescence, such as autophagy-related protein 8 (ATG8) and superoxide dismutase genes, showed altered expression patterns, suggesting that infected leaves age and deteriorate more quickly than healthy ones. Additionally, metabolic changes, including increased lignin production and secondary metabolite accumulation, suggest that the plant is responding to stress by strengthening its cell walls and modifying its defense mechanisms (Tan *et al*. 2025). These findings indicate that phytoplasma infection triggers premature senescence and structural changes as stress responses, leading to leaf size reduction and bushy growth (witches'-broom).

Integrating omics-based and targeted molecular studies enhances understanding of phytoplasma-host interactions, guiding strategies for disease resistance and management in sweet cherry and other crops. Future research should focus on identifying resistance genes, effector targets, and metabolic pathways to develop phytoplasma-resistant crops and targeted disease control interventions.

PHYTOPLASMA TAXONOMY: NOMENCLATURE, CLASSIFICATION, AND IDENTIFICATION

Historical background and challenges in phytoplasma classification

Phytoplasmas were historically misclassified as viruses due to their uncultivable nature. In 1967, researchers identified them as bacteria which were initially referred to as mycoplasma-like organisms (MLOs) due to their resemblance to mycoplasmas, which infect animals (Doi *et al*. 1967). In 1993, the International Committee on Systematic Bacteriology (ICSB) formally designated them as phytoplasmas, emphasizing their role as plant pathogens (International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of Mollicutes 1993).

One of the biggest challenges in phytoplasma research is their lack of cultivability, making identification, classification, and characterization particularly difficult. Early classification attempts were based on symptomatology, host range, insect vector specificity, and serological markers. However, these approaches were inconsistent and unreliable due to the variability in symptom expression and host-pathogen interactions. The situation changed in the 1990s with the introduction of molecular and culture-independent genotypic approaches, which revolutionized bacterial systematics, including the study of both culturable and non-culturable bacteria like phytoplasmas (Wei & Zhao 2022).

Phytoplasma nomenclature

Since phytoplasmas cannot be grown *in vitro*, they are currently accommodated under the genus '*Candidatus* Phytoplasma' (Murray & Schleifer 1994). The first formally recognized '*Candidatus* Phytoplasma' species was '*Ca*. Phytoplasma aurantifolia' in 1995, associated with lime witches'-broom disease (Zreik *et al*. 1995).

For decades, phytoplasma naming was primarily based on 16S rRNA gene sequencing, with a demarcation threshold of 97.5% sequence identity to define species (IRPCM Phytoplasma/Spiroplasma Working Team- Phytoplasma Taxonomy Group 2004). However, as sequencing technologies advanced, whole-genome sequencing (WGS) emerged as a more precise classification method. In 2022, the following new guidelines were introduced for phytoplasma taxonomy: (1) Increase the 16S rRNA sequence identity threshold from 97.5% to 98.65% for species-level differentiation, (2) Introduce whole-genome average nucleotide identity (ANI) with a threshold of 95–96% as a key classification criterion, (3) Incorporate multi-locus sequence analysis (MLSA), using conserved housekeeping genes such as *groEL*, *tuf*, *secA*, and *secY* to distinguish closely related species (Bertaccini *et al*. 2022; Wei & Zhao 2022).

Database-guided phytoplasma classification

Phytoplasmas are primarily classified using the 16Sr group/subgroup system, which is based on restriction fragment length polymorphism (RFLP) analysis of the 16S rRNA gene (Lee *et al*. 1995). This method, developed in the 1990s, allows researchers to group phytoplasma strains into well-defined categories based on genetic similarity. An online tool called *i*PhyClassifier has been developed to facilitate this classification, allowing scientists to automate the process of assigning new phytoplasma strains to existing groups (Fig. 5; Wei *et al*. 2007, 2008b; Zhao *et al*. 2009). *i*PhyClassifier is a user-friendly online tool that allows researchers to (1) assign species under the '*Candidatus* Phytoplasma' system, (2) classify phytoplasmas into 16Sr groups and subgroups, (3) calculate RFLP similarity coefficients, (4) generate virtual gel images for comparative analysis, and (5) compare RFLP patterns with reference strains.

By simply inputting a phytoplasma 16S rRNA gene sequence, users can obtain automated classification results, making phytoplasma taxonomy more efficient and standardized. Using *i*PhyClassifier, researchers have identified emerging and reemerging phytoplasma diseases, improving disease surveillance and classification accuracy.

Currently, phytoplasmas are divided into 37 groups and more than 150 subgroups. Each group typically corresponds to a specific disease and geographical distribution (Wei & Zhao 2022). For example, the 16SrI (Aster Yellows Group) includes phytoplasmas that infect a wide variety of crops, including wheat, lettuce, and tomatoes. However, classification remains challenging, as some phytoplasma strains share high genetic similarity but differ significantly in ecological behavior, host range, and insect vector specificity. The integration of whole-genome sequencing into phytoplasma classification is expected to resolve many of these ambiguities and improve species differentiation.

Phytoplasma identification and diagnostic methods

Since phytoplasmas cannot be cultured, their identification relies entirely on molecular diagnostic techniques. Among the most widely used methods, PCR-based detection remains the standard approach for detecting phytoplasma DNA in infected plant tissues. Conventional PCR is commonly employed to amplify phytoplasma DNA, providing a reliable means of detection (Lee *et al*. 1993). However, when phytoplasma concentrations are low, nested PCR is often used for its enhanced sensitivity,

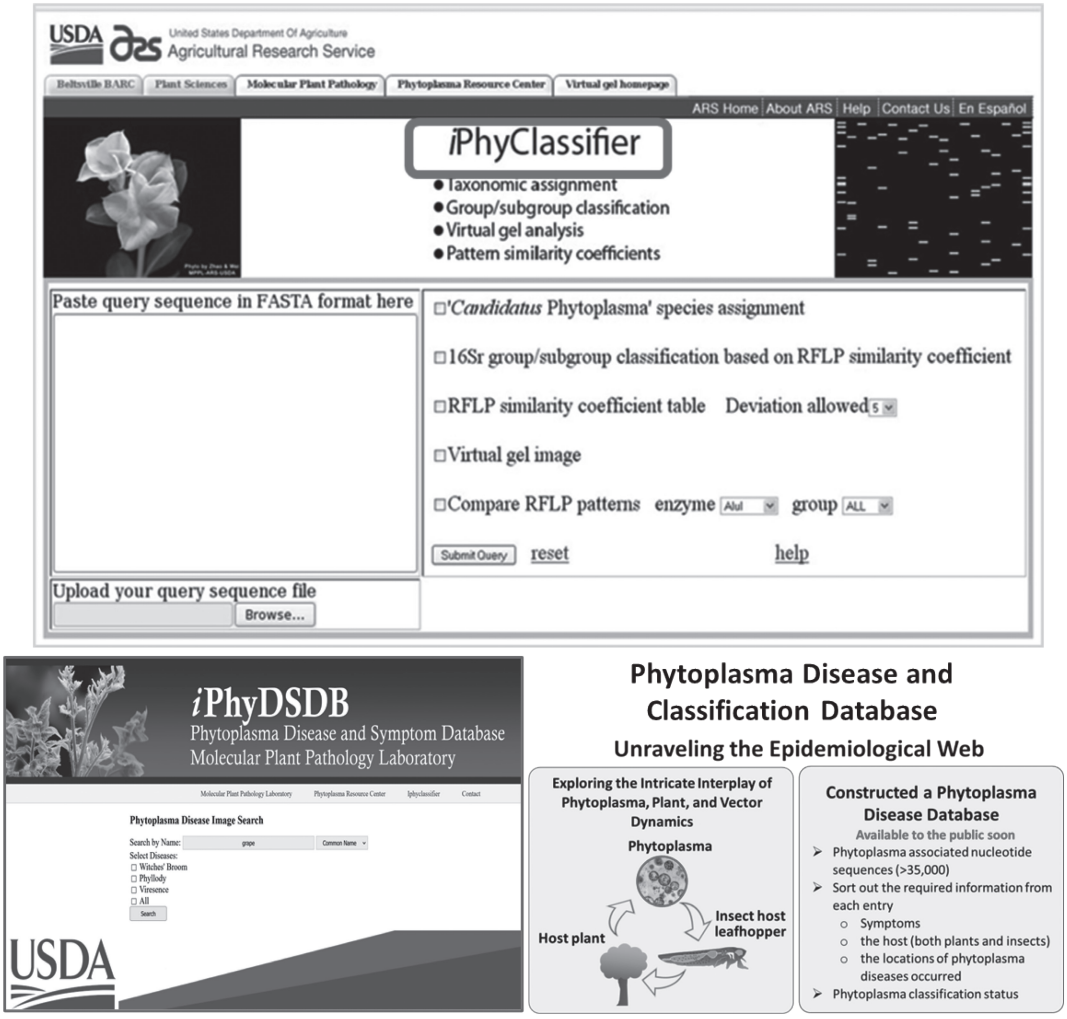

Fig. 5. Database-guided classification, identification, and epidemiological prediction of emerging and reemerging phytoplasma diseases.

enabling more accurate detection in samples with minimal pathogen presence (Gundersen & Lee 1996). More advanced methods, such as PCR, nested PCR, real-time PCR (qPCR, Christensen *et al*. 2004; Wei *et al*. 2004) and droplet digital PCR (ddPCR, Mehle *et al*. 2014), enable quantitative detection, offering deeper insights into disease progression and pathogen load in infected plants.

In addition to PCR-based methods, recent advancements in field-based diagnostics have significantly improved the speed and accuracy of phytoplasma detection. Loop-mediated isothermal amplification (LAMP) has emerged as a rapid and cost-effective technique that does not require sophisticated laboratory equipment, making it suitable for on-site diagnostics (Dickinson 2015). Another cutting-edge technology, CRISPR-based detection, leverages CRISPR-Cas systems to identify phytoplasma DNA with high specificity and accuracy (Wheatley *et al*. 2022). This approach holds great promise for real-time, field-deployable diagnostics, allowing for quicker disease management and intervention in agricultural settings.

AI AND DATA SCIENCE IN PHYTOPLASMA RESEARCH: ENHANCING DISEASE MONITORING

AI-based detection of phytoplasma diseases

Traditional diagnostic methods, such as polymerase chain reaction (PCR), and quantitative PCR, are time-consuming, labor-intensive, and require specialized laboratory equipment, making them impractical for most farmers, particularly in resource-limited settings. As a result, there is a growing need for rapid, accessible, and cost-effective diagnostic solutions. Our group explores an AI-driven system utilizing Convolutional Neural Networks (CNNs) to analyze plant images for early and efficient disease detection, enabling timely intervention for improved crop management (Wei *et al*. 2024b; Wei *et al*. 2025).

A case study was conducted on tomato plants infected by PPT phytoplasma. A comprehensive dataset was compiled, consisting of thousands of images capturing both healthy and infected plants. To ensure the reliability of the model, 20% of the dataset was reserved for testing and validation. Five CNN architectures were trained using transfer learning: four pre-trained models (VGG-16, Google Inception v3, NASNet, and DenseNet201) and one custom model specifically designed for phytoplasma detection. The pre-trained models leveraged weights from ImageNet, which provided a strong foundation for feature extraction and image classification. In contrast, the custom model was developed from scratch to capture unique morphological changes associated with phytoplasma infections. To enhance model robustness and reduce overfitting, TensorFlow was used to implement data augmentation techniques, including image rotation, flipping, and contrast adjustments. These preprocessing techniques helped simulate real-world variations in plant appearances, ensuring that the model generalizes well to diverse conditions (Wei *et al*. 2025).

The pre-trained models exhibited strong performance due to their extensive prior training on large-scale image datasets, allowing them to effectively distinguish between healthy and diseased plants. Among the pre-trained models, DenseNet201 and NASNet demonstrated the highest classification accuracy, demonstrating their ability to recognize subtle visual patterns indicative of phytoplasma infections. The custom model, while requiring more data and training time, provided valuable insights into phytoplasma-specific markers and offered a more tailored approach to disease identification (Wei *et al*. 2025). To enhance diagnostic accuracy and overall reliability, ensemble learning techniques were implemented. By combining the predictions of multiple models using majority and weighted voting strategies, the system mitigated the limitations of individual models and significantly improved classification performance. The ensemble approach effectively reduced false positives and false negatives, ensuring more consistent and dependable results across varied image inputs (Wei *et al*. 2025). Future research should focus on expanding the dataset to include a wider range of plant species and different phytoplasma diseases.

Phytoplasma Disease and Symptom Database (*i*PhyDSDB)

In the meantime, to support farmers and growers in recognizing diseases in their crops, we developed an image and symptom database known as the Phytoplasma Disease and Symptom Database (*i*PhyDSDB, Fig. 5; Wei *et al*. 2024a). This database contains a comprehensive collection of plant symptom images and associated data. It serves as a critical backup tool, allowing users to compare symptoms observed in their crops with those in the database, helping them to identify potential infections early on. By offering this interim solution, we aim to empower farmers and gain recognition while the AI-based system undergoes further development. Ultimately, the combination of the AI

model and this robust database will provide an integrated, scalable approach to managing phytoplasma diseases and safeguarding crop production.

Phytoplasma Disease and Classification Database

A Phytoplasma Disease and Classification Database is under construction (unpublished). This is a searchable database that links each isolated case and provides the users with a comprehensive view, making it possible to quickly compare various phytoplasma diseases from symptoms, host, and classification status. The Phytoplasma Disease and Classification Database is very helpful for early warning of the occurrence of phytoplasma diseases and provides the basis for the formulation of effective prevention and control measures.

In recent years, the introduction of drone technology has transformed phytoplasma research by enabling efficient sample collection from previously inaccessible regions, improving the identification of new phytoplasma strains and enhancing disease epidemiology studies. As plant and insect sampling increases, so will discoveries of novel phytoplasmas, further expanding our understanding of their distribution and impact. Big data analytics has already demonstrated its potential in tools such as *i*PhyClassifier, *i*PhyDSDB, and the Phytoplasma Disease and Classification Database, streamlining taxonomy and disease monitoring. Moving forward, the integration of big data, knowledge networks, and precision agriculture technologies will enhance research depth and improve disease management strategies.

CONCLUSIONS

The combination of top-down multi-omics and bottom-up molecular analyses has advanced phytoplasma research, revealing key molecular mechanisms of pathogenesis and symptom development. Innovations in whole-genome sequencing, molecular detection tools, AI-driven surveillance, and big data analytics continue to refine taxonomy, diagnostics, and disease management. Sustained interdisciplinary collaboration will be crucial for developing effective and sustainable strategies to mitigate phytoplasma-related agricultural loss.

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由上而下與由下而上的植物菌質體研究：致病機制、分類學及診斷技術之進展

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摘要

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植物菌質體為微小且無細胞壁的細菌，可導致毀滅性的植物病害，造成農業重大經濟損失。本文探討植物菌質體的最新研究進展，重點在於其致病機制、分類學以及透過由上而下與由下而上的診斷方法。由上而下的多組學研究 (multi-omics studies)，對於植物菌質體所引起的干擾 (尤其是在糖代謝與賀爾蒙傳導方面) 提供系統層次的理解，揭示其對植物生理之廣泛影響。除此，由下而上的研究策略則剖析了分子相互作用，闡明植物菌質體如何干擾分生組織的歷程、如何調節植物生長模式，以及如何改變植物結構並誘發可作為區辨特徵的危害徵狀。分類學 (taxonomy) 與特徵歸類的分類法 (classification) 的進步改善了物種分化，整合了 16S rRNA 定序、多位點序列分型 (multilocus sequence typing; MLST) 及全基因組定序 (whole-genome sequencing; WGS)，並透過資料庫指導工具提高了分類準確性。基於 clustered regularly interspaced short palindromic repeats-based (CRISPR) 的檢測等尖端診斷技術的發展，顯著提高了植物菌質體鑑定與監測的敏感度、特異性及效率。此外，大數據分析與人工智慧驅動模型的整合，開創了基於影像的症狀識別技術，為疾病監測與監控提供支援。本文整合了關鍵研究成果與技術進步，展示了整合由上而下的系統生物學 (systems biology) 與由下而上的分子分析，如何創新推動植物菌質體的檢測、分類以及永續的病害管理策略。

關鍵詞：分生組織命運偏離、*i*phy 分類系統、人工智慧、組學、花期轉換。

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